REMARKS

Claim Amendment

Claims 1, 2, 22 and 23 have been canceled by this Amendment.

Claims 3 and 6 have been amended to depend from new claim 33.

Claim 8 has been amended to depend from new claim 33 and to make the language consistent with new claim 33.

Claim 17 has been amended to depend from new claim 36 and to specify that the mammalian cell is transfected with the first and second amplified products containing the two cassettes, one with the sense strand and the other with antisense strand of the siRNA molecule. Support for transfection with two cassettes can be found, for example, in paragraphs 52 and 71 of the published application.

Claim 21 has been amended to be consistent with amended claim 17.

New claim 33 has been written to replace claims 1 and 2 and to make the language clear concerning the PCR amplification that is used to amplify the mammalian promoter-containing siRNA expression cassette and to specify the product of the reaction. Support for PCR amplification can be found, for example, in original claim 2 and in Example 2 of the published application. Support for the second primer being complementary to the 3' end of the sense strand can be found, for example, in Example 2 of the published application, i.e., complementary to the last 20 nucleotides of the promoter which are at the '3 end of the sense strand. Since the amplification is a PCR amplification and since the second primer is complementary to the 3' end of the sense strand, the first primer is complementary to the 3' end of the antisense strand. Thus, support for the nature of the first primer is also found, for example, in Example 2 of the published application. Support for the three cycles of the PCR amplification can be found, for example, in original claim 1 and Example 2 of the published application. Support for the product language can be found, for example, in paragraphs 33, 34, 37 and 40 of the published application.

New claim 34 has been added to specify that the amplification product of claim 33 is purified. Support for this language can be found, for example, in paragraph 52 of the published patent application.

New claim 35 has been added to specify that the amplification product of claim 33 is cloned into a cloning vector. Support for this language can be found, for example, in paragraph 47 of the published application.

New claim 36 has been added to specify that two PCR amplifications are performed to produce a first amplified product comprising the sense strand of the siRNA molecule and a second amplified product comprising the antisense strand of the siRNA molecule. Support for this language can be found, for example, in paragraphs 42, 63 and 71 and Figure 1A of the published patent application.

New claim 37 has been added to specify that the amplification products of claim 36 are purified. Support for this language can be found, for example, in paragraph 52 of the published patent application.

New claim 38 has been added to specify that the amplification products of claim 36 are cloned into a cloning vector. Support for this language can be found, for example, in paragraph 47 of the published application.

Applicants submit that none of these amendments constitute new matter, and their entry is requested.

The Present Invention

Claim 33 is directed to an amplification-based method for producing a mammalian promoter-containing siRNA expression cassette. The method comprises (i) adding a double stranded nucleic acid comprising a mammalian promoter to an amplification reaction mixture, wherein the double stranded nucleic acid has a sense strand and an antisense strand and wherein each of the sense strand and antisense strand has a 5' end and a 3' end, wherein the mammalian promoter is capable of transcribing an RNA molecule in mammalian cells. The method also comprises (ii) adding a first oligonucleotide primer to the reaction mixture, wherein the first

oligonucleotide primer is complementary to the 3' end of the antisense strand of the double stranded nucleic acid. The method further comprises (iii) adding a second oligonucleotide primer to the reaction mixture, wherein the second oligonucleotide primer is complementary to the 3' end of the sense strand of the double stranded nucleic acid and wherein the second oligonucleotide comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes (1) either a sense sequence of a double stranded siRNA molecule or an antisense sequence of the double stranded siRNA molecule and (2) a terminator sequence. Finally, the method also comprises (iv) amplifying the double stranded nucleic acid in a polymerase chain reaction amplification comprising (a) annealing the primers to the complementary strands of the double stranded nucleic acid, (b) extending the annealed primers to produce extension products, (c) denaturing the extension products and (d) repeating the polymerase chain reaction amplification steps a sufficient number of times to produce an amplified product comprising the mammalian promoter-containing siRNA expression cassette. The mammalian promoter-containing siRNA expression cassette produced in this method comprises (1) the mammalian promoter, (2) either the sense strand or the antisense strand of the double stranded siRNA molecule and (3) the terminator sequence. It is clear from the language of claim 33 that the primer sequences are complementary to the promoter sequence and are not complementary to the siRNA sense or antisense sequences.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 1-9, 17 and 19-23 under 35 U.S.C. § 112, second paragraph for being indefinite. In essence, the Examiner contends that the use of the terms "one strand" and "other strand" are not distinctly claimed and it is not clear what is meant by the first primer being complementary to the 5' end of the promoter and the second primer being complementary to the 3' end of the promoter. In addition, the Examiner contends that claim 1 fails to include steps or materials for producing a double stranded siRNA molecule. Since claim 1 does not produce a double stranded siRNA molecule, the transfected cell of claim 17 will not contain a double stranded siRNA molecule.

Applicants have rewritten claims 1 and 2 as new claim 33. Claim 33 specifies the primers in the context of the PCR amplification that is performed in claim 33. Thus, the primers are (a) complementary to the 3' end of the sense strand, i.e., the second oligonucleotide primer that contains a sequence encoding the sense or antisense strand of the siRNA molecule and (b) complementary to the 3' end of the antisense strand. The PCR amplification is performed to produce an amplified product comprising the mammalian promoter-containing siRNA expression cassette, in which the mammalian promoter-containing siRNA expression cassette comprises (1) the mammalian promoter, (2) either the sense strand or the antisense strand of the double stranded siRNA molecule and (3) the terminator sequence. Applicants submit that claim 33 and its dependent claims are definite and clear to the skilled artisan, the person to whom the specification and claims are directed.

Applicants have added new claim 36 which is directed to the method of claim 33 in which two PCR amplifications are performed so that two expression cassettes are produced in which one contains the sense strand and the other contains the antisense strand. Claim 17 has then been amended so that the cell is transfected with both of these expression cassettes. Applicants submit that claim 17 and its dependent claims are definite and clear to the skilled artisan, the person to whom the specification and claims are directed.

In view of the above amendments and remarks, Applicants submit that the claims are definite and clear to the skilled artisan. Withdrawal of this rejection is requested.

Rejection under 35 U.S.C. § 103(a)

The Examiner has also rejected claims 1-9, 17 and 19-23 under 35 U.S.C. § 103(a) as being unpatentable over Lee et al. (*Nature Biotechnology* **19**:500-505, 2002) in view of MacFerrin et al. (*Proc Natl Acad Sci USA* **87**:1937-1941, 1990), Lindermann et al. (US 5,958,738) and Livache et al. (US 5,795,715). The Examiner cites Lee et al. for its disclosure of the construction of a U6 promoter-containing expression cassette for expressing the sense strand of a siRNA molecule or the antisense strand of a siRNA molecule in which the siRNA sequence is operably placed downstream from the human U6 snRNA promoter and upstream from the

termination signal sequence (a short stretch of six thymidines). The Examiner also cites Lee et al. for its disclosure that the siRNA sequence (sense strand or antisense strand sequence) is inserted into a transcriptional cassette via restriction enzyme-based digestion and ligation methods and that the U6 promoter-containing siRNA expression vector can be transfected into mammalian cells. The Examiner notes that Lee et al. does not teach that the siRNA expression cassette is produced by a PCR-based amplification method. The Examiner cites MacFerrin et al. for its disclosure of a PCR amplification-based method for producing a promoter-containing, double-stranded oligonucleotide expression cassette, termed expression-cassette polymerase chain reaction (ECPCR). The Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to make the expression cassette comprising the U6 promoter-siRNA sequence termination signal sequence insert of Lee et al. by utilizing the ECPCR protocol of MacFerrin et al. Applicants traverse this rejection.

Applicants note that the primary reference, Lee et al., was published in May 2002. Applicants direct the attention of the Examiner to the Rule 131 Declaration that was filed on 9 July 2007 and its Exhibit A that was filed on 10 July 2007 in the present application. This Rule 131 Declaration establishes a date of invention of at least prior to 15 April 2002, which is before the publication date of Lee et al. Thus, it is submitted that Applicants have sworn behind Lee et al., thereby removing it as prior art. In view of the earlier date of invention and the removal of Lee et al. as prior art, Applicants submit that the claimed invention is not rendered obvious by the prior art.

In view of the above amendments and remarks, Applicants submit that the present invention is not rendered obvious by the combination of Lee et al., MacFerrin et al., Lindermann et al. and Livache et al. Withdrawal of this rejection is requested.

Conclusion

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and reconsideration of the instant application and early notice

of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By /Jeffrey L. Ihnen/

Jeffrey L. Ihnen
Registration No. 28,957
Attorney for Applicants
1425 K Street, N.W., Suite 800
Washington, D.C. 20005
Telephone: (202)783 6040
Facsimile: (202)783-6031

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